NBPDS Genotyping External Quality Assessment Protocol
Approved July 2011

Create a standard protocol using control samples for external quality assessment (EQA). The primary objective of establishing EQA is to ensure that each lab actively involved in genotyping NBDPS samples is proficient in their respective genotyping techniques independent of the source material or extraction procedure.

EQA Samples: Blood-Buccal Trios

- Composition of Blood-Buccal Trio Samples:
  - Recruit a total of 6 parent-offspring trios (n=18) following appropriate human subjects requirements. Obtain 4 buccal specimens per participant using the dry brush method and two whole blood specimen from each participant.
  - Ship the specimens (n=108) to the NBDPS Central Lab where DNA will be isolated and quantified, Mendelian inheritance will be checked using a microsatellite panel, DNA concentrations will be normalized, and aliquots will be prepared in micropackaging vials labeled with only a CDC unique (ASTRO) ID. (n=36 total samples; 18 blood + 18 buccal)
  - Labs using lower throughput platforms (e.g., TaqMan, Pyrosequencing): Blinded DNA aliquots with concentrations of 5ng/µl from buccals (DNA yield= 100ng) and whole blood (DNA yield= 100ng) and DNA negative controls will be sent to each genotyping lab.
  - Labs using higher throughput platforms (e.g., Illumina, Affymetrix): Blinded DNA aliquots with higher concentrations and yields, dependent on assay requirements, will be sent to each genotyping lab
  - All labs will be responsible for performing whole genome amplification (WGA) on the samples using their preferred methodology if they propose to perform WGA on NBDPS samples.
  - The same genotyping methods/platforms that will be used with NBDPS samples will be used with EQA samples.

- Use of Blood-Buccal Trio Samples:
  - Labs using lower throughput platforms (e.g., TaqMan, Pyrosequencing): The samples will be used prior to initiating NBDPS genotyping with annual re-assessment to test 2 - 5 SNPs selected by GAWG members. SNPs chosen will include those the investigator is proposing to perform on NBDPS samples and those assayed by more than one lab when possible.
  - Each genotyping lab will be required to genotype one SNP that is agreed upon by the GAWG.
  - Laboratories using high throughput platforms (e.g., Illumina, Affymetrix) with NBDPS samples will include one SNP that is agreed upon by the GAWG to genotype samples from one blood-buccal trio family (6 gDNA and 6 WGA products). In addition to results from the one agreed-upon SNP, labs should also report results from all variants tested. The samples will be used prior to initiating NBDPS genotyping and will be required one time per lab per project.

- Paired Blood-Buccal Trios Will Allow:
  - Intra-lab comparison of results from blood compared to buccals and to WGA products
  - Intra-lab verification of genotype accuracy by Mendelian inheritance
  - Inter-lab comparison for results of one SNP each lab will genotype
  - Inter-lab comparison of SNPs labs assay in common when possible

EQA Samples: Pre-Characterized (Polymorphism Discovery Resource from Coriell)

- Composition of Pre-Characterized Samples:
  - Determine which gene variants approved for NBDPS are listed on the website for the pre-characterized samples.
  - Purchase pre-characterized sample set or subset.
  - Coriell ships 96-well plates to each investigator that contain 86 PDR samples, 4 duplicate PDR samples, 2 water controls, and 4 empty wells for internal genotyping controls. Investigators are blinded to samples in all wells and will genotype the samples for SNPs that they plan to genotype in the NBDPS. The same genotyping methods/platforms that will be used with NBDPS samples will be used with EQA samples. Results will be reported back to CDC and compared to results from other labs and the published results.

- Use of Pre-Characterized Samples:
  - Pre-characterized samples will be used prior to initiating NBDPS genotyping with annual re-assessment to test 2 - 5 SNPs selected by GAWG members. SNPs chosen will include those the investigator is proposing to perform on NBDPS samples and those assayed by more than one lab when possible.
  - Pre-characterized samples will not be included in arrays from high throughput platforms (e.g., Illumina, Affymetrix).

- Pre-Characterized Samples Will Allow:
  - Comparison to published third-party results
  - Inter-lab comparison of SNPs labs assay in common

Standards Required to Pass EQA:

- 90% genotyping call rate per gene variant
- 99% concordance between successful genotyping data for:
  - paired blood and buccal DNA
  - gDNA and WGA product
  - inter-lab SNPs assayed in common
  - pre-characterized DNA and published third-party results
- No results reported for negative controls
- Genotyping results of trios consistent with Mendelian inheritance

- If inter-lab results for SNP assays performed in common are discordant, results from SNP assays performed on pre-characterized samples will be compared to third party published results to determine if a lab needs to identify and resolve problems.
- If a genotyping lab does not pass EQA standards, they must discontinue all genotyping and repeat EQA. If the genotyping lab does not pass EQA standards a second time, no manuscripts will be completed until the problems are identified and resolved.

Additional Items:

- Choose one gene variant that all genotyping labs agree to assay: MTHFR C677T
- Results reported to CDC are final (e.g., if errors are made transcribing data to results template and the data do not meet the standards required to pass EQA, the lab must repeat EQA).